

THE GERM CELL CYCLE IN THE TREMATODE,
PARAGONIMUS KELLICOTTI WARD

PIN-DJI CHEN

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE UNIVERSITY
OF MICHIGAN

Reprinted from TRANSACTIONS OF THE AMERICAN MICROSCOPICAL SOCIETY
Vol. LVI, No. 2, April, 1937



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INTRODUCTION

In the complicated life history of digenetic trematodes several generations and two or more host species are involved. The list of generations, as usually given, includes the adult, i.e., the egg-producing generation, occurring in a vertebrate; the miracidium-sporocyst generation, at first a free swimming embryo, and later a sac-shaped body parasitic in a mollusc; followed in this same host by one or more generations of sporocysts or rediae. Cercariae which are the larvae of adults develop within the last generation. Various studies have shown that within the miracidium and its successor generations in the mollusc there are certain large cells, germ cells, from which the next generations develop. Thus a considerable multiplication in the number of larvae is brought about within the mollusc through the several generations.

Contemplation of these and related facts has given rise to much speculation and has furnished the impetus to many investigations since Steenstrup (1842) first drew attention to the complexity of the trematode life history. Despite the work of many investigators whose results are not in accord, certain questions remain without satisfactory solutions. Some of these questions are: What is the origin of the germ cells in the miracidium-sporocyst and in subsequent generations? What is their early and subsequent history in each of these generations? How does the multiplication of individuals take place in each generation?

Examination of the literature shows conflict of views on these questions, too serious to be resolved without more data. It also shows that, although numerous opinions and theories have been presented, the number of well planned and thorough-going attacks upon the problem of the germ cell cycle in digenetic trematodes are few. Many of the observations upon which theories have been based were incidental to other investigations and might well be called by-products.

In undertaking this investigation I have had no thought of furnishing a complete solution of the problem of the germ cycle of digenetic trematodes. Rather, it was my belief that an extensive and intensive study of the origin and subsequent history of the germ cells in every generation of the life history of a single species might be a contribution toward the solution of this problem.

When Ameel in this laboratory worked out and reported (1934) the complete life history of *Paragonimus kellicotti* Ward in Michigan and re-

* Contribution from the Department of Zoology, University of Michigan.

vealed the methods required for securing all the desired stages in suitable quantities, this species was chosen for cytological study.

Spermatogenesis and oogenesis in the adult *Paragonimus* were studied first so as to get a fundamental knowledge of the nuclear changes and maturation phenomena of the germ cells. The development of the fertilized ovum inside the egg has been followed from cleavage until the miracidium-sporocyst is completely formed in order to secure the facts concerning the origin, in this stage, of the germ cell from which subsequent generations are derived. Likewise in the redial and cercarial generations development was followed with great care from a single germ cell to the formation of the adult in order that nothing of importance in the development of the germ cells in these stages might be overlooked.

To accomplish this object much material has been used for the study of the germ cells and their progeny in a single stage of development in every generation of the life cycle. Careful, and often tedious, observations have been made on every cell in all the serial sections of the different stages. From this intensive, as well as extensive, study it has been possible to secure a clear conception of the germ cycle in this species of trematode.

HISTORICAL SURVEY

Brooks (1930) classified into five groups the many theories concerning the germ cell cycle in trematodes, and these were reclassified by Cable (1934) to whose work the reader is referred for a detailed review. These theories are here briefly stated.

According to the theory of metagenesis the life history is divided into a sexual and an asexual generation. Steenstrup (1842, 1845) applied this theory to trematodes as well as to coelenterates and tunicates. It was supported by Moulinié (1856), Pagenstecher (1857), Wagener (1866) and Biehringer (1884).

Heterogony is the name given by Grobben (1882) to a type of life cycle in trematodes in which a bisexual generation alternates with parthenogenetic generations. The germ cells in the latter are considered to be ova which give off polar bodies and develop parthenogenetically. This theory was supported by Reuss (1903), Haswell (1903), Tennent (1906), Cary (1909), Ssinitzin (1911, 1931, 1933), Faust (1917), and Sewell (1922). Opposed to this view are such investigators as Looss (1892), Coe (1896), Rossback (1906), Brooks (1930) and Cable (1934) who failed to find polar bodies.

In paedogenesis reproduction takes place in the larval stage. This theory, according to Brooks (1930), is no longer considered as a tenable explanation of the life history of the trematodes, and Cable (1934) did not even mention it.

The theory of extended metamorphosis was proposed by Leuckart

(1879), accepted by Balfour (1880), and restated in English by Leuckart (1886). The sporocyst, redia and cercaria were thought by Looss (1892) to be homologous, and regarded as parts of an extended metamorphosis. Concerning this Brooks (1930) wrote, "Leuckart argued that if a series of different developmental phases may contract into a single continuous process, then conversely, the latter can also spread itself out into a number of such phases."

The theory of germinal lineage either with or without polyembryony has been applied to the trematode life cycle by a number of investigators. According to it the germ cells persist through generations of the life history; they multiply by the method of polyembryony, and give rise to the bodies of succeeding generations which house the germ cells. The foundation of this theory was laid by Leuckart (1879), but it was modified and added to by Thomas (1883), Schwarze (1886), Dollfus (1919), Kathariner (1920), Brooks (1928, 1930) and Cable (1934).

The theory of polymorphism was presented by Woodhead (1931). According to it spermatozoa and ova are produced in the sporocyst and redia where the process of reduction and fertilization occurs as well as in the egg-laying adult. Reproduction in all stages of *Bucephalus* is considered as bisexual.

Concerning the diversity of observations on this subject, Cable (1934) said that no single interpretation of the germinal development in trematodes may be applied to the group as a whole. Many observations have not been adequately supported by subsequent work. Further work is necessary before it can be determined whether a single interpretation of germinal development will prove adequate, or whether several theories must be employed.

ACKNOWLEDGMENTS

The writer is deeply indebted to Professor George R. La Rue of the Zoology Department, University of Michigan, for his kindly interest and very helpful suggestions concerning this investigation which was made under his direction.

The writer also desires to express her thanks to Professor A. E. Woodhead for his encouragement and aid in securing material for this study; to Dr. Donald Ameel for useful information concerning the experimental infections; to Dr. Helen F. Price for help in collecting materials, and to various other persons for their kindly assistance.

MATERIALS AND METHODS

Adult: Adult worms from the lungs of minks and cats were used, but the best specimens for the study of oogenesis and spermatogenesis were obtained from experimentally infected cats killed about four months after

infection. These worms were relatively larger than those from minks and they could be taken at the proper degree of sexual maturity.

Adult worms were fixed in such fixatives as Gilson's, Bouin's, and Flemming's solutions, with or without acetic acid, but Bouin's was used for most specimens. As a protection against damage to delicate cells during and after sectioning, tissues were doubly infiltrated with celloidin and paraffin. Serial sections were cut in thicknesses ranging from five to ten microns, but those of seven or eight microns were the best for study. Flemming's triple stain and Heidenhain's iron hematoxylin without counter stain were used, and the latter gave better results for chromosome study. Some smears of the testis were made for the study of the groups of germ cells.

Eggs: Eggs were collected either from the water in which the infected lungs were washed, or from the adult worm by teasing the uterus. Washed eggs were incubated at 27°C. Eggs used for sectioning were taken after two days of incubation, and others at intervals of a few days until hatching. On account of the smallness of the eggs and the hardness of the shell, a few drops of egg albumen were used to make the eggs adhere in a mass when fixed. Later they were doubly infiltrated. Eggs so prepared were serially sectioned seven microns thick. Flemming's triple stain and iron hematoxylin without counter stain were used. Both stains are good for the purpose.

Miracidium: Miracidia after hatching were fixed in Bouin's fluid and some were sectioned; others were mounted whole. Mayer's haemalum, Flemming's triple stain and iron hematoxylin were used.

Sporocyst, redia and cercaria: The generations studied were sectioned within the tissues of the experimentally infected snail host, *Pomatiopsis lapidaria*, thereby avoiding much distortion caused by handling. In order to get a graded developmental series a number of experimentally infected snails were crushed daily and fixed in Bouin's solution. After double infiltration the tissues were serially sectioned seven microns thick, and stained with iron hematoxylin which proved to be the best stain for the study. Flemming's triple stain is good for studying young sporocysts up to five days of age because it differentiates the muscle cells and body cells clearly from the germ cells. Observations were made with an oil immersion objective and $\times 15$ ocular, and illumination was secured from a Spencer high power lamp with a 500 watt bulb. All the drawings were made with the aid of a camera lucida.

OBSERVATIONS

Adult: The germ cells in the adult were first studied in detail in order to get a basic knowledge of the chromosomes, maturation, and other germ-cell phenomena of this species. It was believed that the information so

gained would simplify the interpretation of the phenomena encountered in the study of germ cells in the other generations. This study then began with the germ cells in the testis.

SPERMATOGENESIS

The male germ cells differ in size, general appearance, and position with reference to the testicular wall near which the spermatogonia form a distinct zone. Spermatocytes, spermatids, and spermatozoa are more centrally located. Near the tunica and sometimes scattered through the mass of testicular tissue are a few masses of numerous small cells with scanty but granular cytoplasm and clear nuclei, each containing a small spherical nucleolus. These cells may be degenerating spermatogonia which may serve as nutritive material for the germ cells.

Spermatogonia: Primary spermatogonia are the least differentiated cells near the periphery of the organ (fig. 1). They average about 4.5μ in diameter and have a relatively large nucleus placed toward one side of the cell and very little cytoplasm. The resting nucleus contains one or two nucleoli and the finely granular chromatin. A primary spermatogonium grows, nearly doubling in size, and its protoplasm then is relatively abundant. As division approaches, the nuclear membrane disappears; the spindle forms and the chromatin forms a spireme arranged in the equatorial plane (fig. 2). Segmentation of the spireme gives rise to chromosomes which split during division. The two daughter cells are secondary spermatogonia which remain connected by a protoplasmic strand (fig. 3). Both cells grow and then divide forming four cells which may be called tertiary spermatogonia (figs. 4, 4a). These four cells are connected by cytoplasmic strands.

These groups begin now to move toward the center of the testis, though some of them are not yet entirely free from the tunica. Tertiary spermatogonia grow rapidly and the division (fig. 4) which follows resembles that in the other spermatogonia. The centriole at each pole divides into two. The normal chromosome number is sixteen. In division the chromosomes split, sixteen going to each daughter cell (fig. 5 two sections of one cell). Through this point the divisions are all equational. Division of the cells in a group of four tertiary spermatogonia gives rise to a group of eight cells whose subsequent history shows them to be primary spermatocytes. Only four or five members of a group of eight can be illustrated in one section (fig. 6).

Spermatocytes and reduction division: Primary spermatocytes are connected with each other by long, slender protoplasmic strands which appear to radiate from a center. The free end of each cell is enlarged, rounded and contains a spherical nucleus. The cytoplasmic strands contain heavily stained fibers and granules. These are best demonstrated in smear preparations where cytoplasmic strands remain entire.

Synapsis and loop formation of chromatin occurs before division of the

primary spermatocytes. Then the chromosomes are formed (fig. 6). The homologous chromosomes separate from each other and each daughter cell gets eight (fig. 7). A similar reduction division affects all eight primary spermatocytes in a group at the same time, thus producing sixteen secondary spermatocytes which are connected by cytoplasmic strands. So far in every division the centriole at each pole divides into two.

At the broad distal end of the newly formed secondary spermatocyte chromosomes are crowded into a single crescent-shaped mass which soon becomes less compact and the nucleus enters a resting stage. Later by mitotic division (figs. 8, 10) the secondary spermatocytes give rise to spermatids. In this division the centriole at each pole does not divide again but remains as a single body.

Spermatids and spermatozoa: The five divisions described above result in the formation of thirty-two spermatids held in one cluster by protoplasmic strands. The spermatids are smaller than the spermatocyte because increase in the number of the cells has not been accompanied by growth of cytoplasm. Each spermatid has eight chromosomes (fig. 9), resembling those in the spermatocyte but smaller. Fibers and granules are present in the protoplasmic strands.

The first noticeable change in the transformation of the spermatids into spermatozoa is seen at the tip of the rounded end of the cell where a protrusion is formed. According to Severinghaus (1928) in *Schistosoma japonicum* a sheet of chondriosome material pushes out in the bulging region and begins to form the tail sheath. The nucleus is still spherical and the chromatin is in the form of a much-coiled thread (fig. 11).

Later the nucleus becomes elongated (fig. 12) and its nuclear material fills the tail part which stretches far beyond the mass of cytoplasm. Cable (1931) thought that the cytoplasm took no part in the formation of the tail in *Cryptocotyle lingua*. In *Paragonimus*, because of the smallness of the spermatids and spermatozoa, it is very difficult to see whether the cytoplasm partakes in the formation of the tail. During this stage the central cytoplasmic mass in which the spermatids are embedded shrinks and loses its original appearance; the cytoplasmic strands fuse more or less and the boundaries of the cells become confused. The resulting mass of cytoplasm is large and irregular with darkly stained, elongated nuclei embedded in it.

Soon each nucleus becomes thread-like with condensed chromatin (fig. 57). This is transformed into the spermatozoon which is a slender, homogeneous, darkly stained body with a head part and a very long slender tail, more than ten times as long as the head part. After the spermatozoa get free the cytoplasm remains as a residual mass. The freed spermatozoa usually stay together in a bundle for a time, but soon they separate and swim in the spermatic fluid. When in motion the head part stretches, so it does not appear as plump as when at rest.

EXPLANATION OF PLATES

All drawings were made with the aid of a camera lucida. The value of the scale is always 0.01 mm. Figs. 1 to 24 inclusive are drawn to the scale shown beside fig. 8. Figs. 25 to 44 inclusive have the same scale as shown beside figs. 25 and 31. Figs. 45-57 inclusive have their own scales.

Abbreviations:

bl—excretory bladder
g—germ cell
gb—germ ball
gp—genital primordium
i—gut

p—propagatory cell (stem cell)
ph—pharynx
sp—sperm
vm—vitelline membrane

PLATE I

FIG. 1. Primary spermatogonium.

FIG. 2. Primary spermatogonium in division. Metaphase.

FIG. 3. Two secondary spermatogonia.

FIGS. 4 and 4a. Tertiary spermatogonia in division. Fig. 4a represents the next section of two spermatogonia.

FIGS. 5 and 5a. Primary spermatocyte, two sections of one cell. The other seven cells in the group of eight are not shown.

FIG. 6. Primary spermatocytes in division. Anaphase. Only four of this group of eight cells are shown.

FIG. 7. Reduction division in primary spermatocytes. Telophase.

FIG. 8. Secondary spermatocytes. Only five cells in the group of sixteen cells are shown.

FIG. 9. A daughter star during the division of secondary spermatocyte.

FIG. 10. Secondary spermatocytes in division to form spermatids. Two cells in metaphase, others in telophase.

FIG. 11. Spermatid. Only one cell of the group of thirty-two is shown.

FIG. 12. Elongation of spermatids to form spermatozoa.

FIG. 13. Oogonium in ovary.

FIG. 14. Growing oocyte with chromatin-loops formed.

FIG. 15. Growing oocyte with chromatin-loops broken up.

FIG. 16. Oogonium in division. Anaphase.

FIG. 17. Oocyte fully grown.

FIG. 18. Oocyte in oviduct being penetrated by spermatozoon. The chromatin material of the oocyte forming loops.

FIGS. 19 and 19a. Section of oocyte showing chromatin-loops released from nucleolus to form chromosomes; the centrosomes appearing and nuclear membrane beginning to disappear.

Fig. 19a shows the next section of the same oocyte showing spermatozoon in the cytoplasm. Yolk cells surrounding the oocyte are omitted here and in later drawings through development of miracidium.

FIGS. 20 and 20a. Conjugation of the chromosomes in oocyte when spindle is well formed. Fig. 20a is the next section showing the spermatozoon.

FIG. 21. Ovum. First maturation division taking place in uterus, after the egg shell is well formed.

FIG. 22. Ovum. Second maturation division. Metaphase. First polar body lies near the oocyte.

FIG. 23. Ovum. Formation of second polar body.

FIG. 24. Ovum showing female and male pronuclei.

FIG. 25. Fertilized ovum after fusion of male and female pronuclei.

FIGS. 26a, b, c. Comparison of germ cells (*g*) which will form respectively cercaria, second generation redia, and first generation redia. Note ectosome in each.



PLATE I

OÖGENESIS

The germ cells in the ovary are graded as to size, general appearance and position with reference to the wall of the ovary. The least differentiated germ cells, the oogonia, are the smallest cells with little cytoplasm and darkly stained nuclei. These cells are crowded into the peripheral zone of the ovary while more differentiated cells, the oocytes, with much more cytoplasm are more centrally located. The peripheral zone packed closely with small cells with little cytoplasm appears darkly stained in sections while the central portion appears more lightly stained.

Oogonium: The cells located near the ovarian membrane are chiefly resting oogonia. Each has a spherical nucleus with two nucleoli embedded in the chromatin network (fig. 13). At the close of the growth period of the oogonium the chromatin forms a fine coiled spireme which is arranged in the equatorial plane after the spindle forms. The sixteen chromosomes formed by the segmentation of the spireme split and in division sixteen chromosomes pass to each daughter cell (fig. 16). These cells are oocytes.

Oocyte: The small newly formed oocyte grows and its chromosomes assume the form of chromatin granules arranged in a coiled thread which in the synapsis stage becomes doubled. The coiling becomes looser and the double thread forms into loops with both ends attached to the nucleolus which is located at one pole (fig. 14). Then the loops break up (fig. 15) and the chromatin granules of the loops diffuse. The nucleolus now is central and the nuclear membrane is well-formed. The diffused chromatin granules group irregularly in the nucleus, and soon disperse. When the oocyte reaches its full growth the chromatin granules become very fine (fig. 17). A full grown oocyte has a diameter several times that of the oogonium; the nucleus is large and clear with a large heavily stained nucleolus. The cytoplasm accumulates mostly at the opposite poles of the cell making the oocyte spindle-shaped. Many rounded darkly stained granules, like those found by Schellenberg (1911) in the oocyte of *Fasciola hepatica* and called yolk granules by him, lie in the cytoplasm. Similar granules were found in *Asplanchna priodonta* and called "Membrankörper" by Nachtwey (1925). These oocytes are now in a resting stage; growth to full size occurs and they move into the oviduct.

Penetration of spermatozoa: As soon as the full grown oocyte enters the oviduct it is met by spermatozoa, one of which coils around it. The head penetrates into the cytoplasm of the oocyte by the time the two cells reach the ootype where they are surrounded by the vitelline cells. Wassermann (1913) saw some eggs of *Zoogonus mirus* with polyspermy. In *Paragonimus* several sperms may try to enter but only one has been seen within the oocyte. After penetration, the tail of the spermatozoon becomes shortened and is gradually drawn into the head part within the oocyte. Goldschmidt (1908) found the same condition in *Dicrocoelium lanceatum*.

Maturation division: Following penetration of the spermatozoon the oocyte changes materially in preparation for the maturation division which occurs inside the egg whose shell is now well formed. This change consists in the formation of leptotene threads and then loops such as occurred during the growth of the oocyte. The exact number of the loops is difficult to determine (fig. 18), but sometimes when the loops are free from the point of attachment eight can be clearly seen (fig. 19). Schellenberg (1911) found a similar reduction in the number of loops in a corresponding stage in *Fasciola hepatica*, as did Kemnitz (1913) in *Brachycoelium salamandrae* and Lindner (1914) in the spermatocytes of *Schistosoma haematobium*.

The loops are twisted and entwined, then become free from the point of attachment. When free they arrange themselves near the nuclear membrane which now begins to disappear, a process which often starts in one region and progresses over the whole surface. Two centrosomes indistinctly seen before the penetration of the spermatozoon, now appear on the periphery of the cell near the locus of the disappearing nuclear membrane (fig. 19). The appearance of the centrosomes is so timed as to suggest the inference that their activity has something to do with the disappearance of the nuclear membrane. The centriole of each centrosome has already divided into two, hence four centrioles are arranged in a row with a clear area around them.

The chromatin loops now free from the nucleolus, contract and shorten into compact chromosomes lying closely in pairs (fig. 20). The chromosomes differ in shape and size, and often appear as rods, clubs, or spherical bodies. Sometimes they are slender, sometimes contracted and thicker. In this stage the darkly stained, rounded, cytoplasmic granules become smaller and stain more diffusely. Later during the first and second maturation divisions the cytoplasm has a homogeneous appearance with fine granules.

The centrosomes, closely approximated earlier, now separate farther; the centrioles enlarge; the spindle appears and the nuclear membrane disappears. By this time the once thread-like spermatozoon has contracted into an oval compact mass in the cortex of the ovum outside the spindle (figs. 20a, 21).

First polar body: Segregation of the members of the paired chromosomes in the ovum sometimes occurs before the nuclear membrane entirely disappears. This first division results in the formation of the ovum and the first polar body. The latter moves away from the ovum and disappears when the second polar body is given off.

Second polar body: Immediately the spindle for the second polar division appears (fig. 22) and the eight chromosomes arrange themselves in the equatorial plane. The chromosomes split, eight going to each pole (fig. 23). The second division results in the formation of the matured ovum

and the second polar body. During the process the centrioles, left in the ovum after the first maturation division, pass to each pole without dividing as they do in the earlier stage.

Pronuclei: During formation of the second polar body the compact spermatozoon becomes swollen with fluid from the cytoplasm of the ovum and expands to form a vesicular nucleus with chromatin granules. This is the male pronucleus. At the same time the chromosomes of the matured ovum group together to form the female pronucleus (fig. 24).

Discussion: Montgomery (1904) from his study of maturation phenomena concluded that the first maturation division is one of reduction in which entire univalent chromosomes separate from each other. In the maturation period before the first mitosis, half the normal number of chromosomes is in view because of conjugation. Schubmann (1905) in *Fasciola hepatica*, Goldschmidt (1908) in *Dicrocoelium lanceatum*, and Kemitz (1913) in *Brachycoelium salamandrae* found that the first maturation division was one of reduction. This is true of *Paragonimus*.

Kathariner (1904) in *Gyrodactylus elegans* and Goldschmidt (1908) in *Dicrocoelium lanceatum* found what they interpreted to be yolk nuclei in the cytoplasm of the egg. Gille (1914) stated that in *Gyrodactylus elegans* aborted egg cells in the ovary provide nourishment for the oocytes. Schubmann (1905) and Schellenberg (1911) stated that the oocytes remaining connected with the ovarian wall by protoplasmic stalks were thought to have the function of conveying nourishment to the oocytes. In the ovary Schubmann also found oocytes that break down to form nutritive material. Cable (1931) in *Cryptocotyle lingua* found no degenerate cells or granules which may be interpreted as nutritive material. He thought that the nourishing material was the body fluid coming through the mesenchyme. In *Paragonimus* the nourishment for the oocyte may come through the wall of the ovary, because no aborted egg cells have been seen. Species may vary in this respect.

Goldschmidt (1905) thought that during formation of the pronucleus the nucleolus may come from the big single karyomerite when the other paired karyomerites are going to form chromosomes. Gille (1914) believed that the nucleolus participated in chromosome formation, but Schellenberg (1911) did not agree with him. In *Paragonimus* the nucleolus is persistent and disappears only after the last chromosomes are formed. Therefore, in *Paragonimus* the nucleolus seems to take no part in the formation of chromosomes.

Halkin (1902) in *Polystomum integerrimum* found that the attraction sphere in the second maturation division was different from that of the first because it was constituted of a clear zone without trace of rod-like "corpuscules centraux." Goldschmidt (1902) thought that the centrosome may come from the large central karyomerite because he saw no centro-

some near the sperm nucleus. He (1905) found too that in the second spindle of *Zoogonus mirus* the centriole was spherical instead of rod-like. The centrosomes at the two poles are of unequal size, so in result the cell is unequally divided. In *Paragonimus* the centrosomes are of equal size. The centrioles are patent through most of the stages. As the formation of karyomerites has not been seen in *Paragonimus*, it is difficult to determine whether the centrosomes originate from the karyomerite.

Halkin in *Polystomum integerrimum* wrote that the pronuclei are formed by fusion of independent vesicles. So at the beginning the pronuclei were lobed. Similar observations have been made by other investigators. In *Paragonimus* during the formation of pronuclei vesicles form and may fuse before the stage of loosening of the chromatin mass.

The chromosome number in trematodes varies. Halkin (1902) found ten chromosomes in *Polystomum integerrimum*, but Goldschmidt (1902) found only eight in the same species. Kathariner (1904) found eight chromosomes in *Gyrodactylus elegans*, but Gille (1914) found twelve in the same species. Goldschmidt (1908) found ten chromosomes in *Zoogonus mirus*, and twenty chromosomes in *Dicrocoelium lanceatum*. In *Fasciola hepatica* Schellenberg (1911) found twelve chromosomes; in *Brachycoelium salamae* von Kemnitz (1913) found twenty chromosomes; in *Distomum turgidum* Levy (1914) found eighteen chromosomes. Lindner (1914) found fourteen chromosomes in the male of *Schistosoma haematobium* and sixteen in the female, and Severinghaus (1928) reported the same numbers in *Schistosoma japonicum*. Cable (1931) found twelve chromosomes in *Cryptocotyle lingua*, and Woodhead (1931) the same number in *Bucephalidae*. *Paragonimus* has sixteen chromosomes.

The stages of spermatogenesis in *Paragonimus* resemble those described by Dingler (1910) for *Dicrocoelium lanceatum*, and by Cable (1934) for *Cryptocotyle lingua*. In the maturation division pseudo-reduction or conjugation of chromosomes occurs just as was found by Schellenberg (1911) in *Fasciola hepatica*, and by Cable (1934) in *Cryptocotyle lingua*.

Union of pronuclei: It is to be remembered that the ovum is surrounded by the yolk cells, which are omitted from the drawings. Fusion of the two pronuclei begins after the egg has left the body of the worm. The fusion nucleus (fig. 25) is large and contains two large nucleoli. Its chromatin granules are darkly stained and arranged in a radial pattern about the nucleoli; its cytoplasm is filled with granules. Cleavage takes place only after the egg has left the host.

Cleavage: In the division of the ovum the mitotic figure is rarely seen. The first cleavage yields two cells differing in size and appearance (fig. 27a). The cytoplasm of the smaller cell contains many coarse and deeply stained granules (27a_p) which are arranged somewhat radially about a single, centrally located nucleolus like the chromatin granules in the early un-

divided ovum. A dark globular body thrusts outward against the nuclear membrane forming a slight bulge. This may be the "Membrankörper" mentioned by Nachtwey (1925) in *Asplanchna priodonta*, or a "germ cell determinant" which Wilson (1925: 322) stated has "been supposed to come originally from the nucleus."

The larger cell of the first cleavage contains fewer granules and both its cytoplasm and nucleus stain lightly. Ishii (1934) in the eggs of *Fasciolopsis buski* found a stage with two cells corresponding to the small and large cell here described in *Paragonimus*. He called the small cell "propagatory cell," and the large one "ectodermal cell." These names can properly be applied to these cells in *Paragonimus*. The propagatory cell has also been called "stem cell" by some cytologists.

The second cleavage (fig. 28a) affects only the large cell and results in two small cells with indistinct boundaries which are destined to produce much of the soma of the miracidium. The nucleus of one of the two cells contains relatively few lightly stained granules and a large nucleolus, while the nucleus of the other cell contains many darkly stained granules and a small nucleolus. The propagatory cell has remained in contact with the two smaller cells, and by this time the darkly staining body seen lying against its nuclear membrane in the two celled stage has disappeared. The

EXPLANATION OF PLATE II

- FIGS. 27a, b, c. Comparing first cleavage of (a) fertilized ovum, "Membrankörper" in propagatory cell (p); (b) of first generation redia; and (c) of second generation redia showing ectosome in the propagatory cell.
- FIGS. 28a and b. Comparison of second cleavage of (a) fertilized ovum; and (b) of first generation redia. Propagatory cell in each unchanged.
- FIGS. 29a and b. Comparison of third cleavage of fertilized ovum (a) and of first generation redia (b). Propagatory cell in each unchanged.
- FIGS. 30a, b, c. Comparison of young embryo of miracidium (a) showing a cell separating from the main cell group to form the vitelline membrane (vm); of second generation redia (b); of first generation redia (c) showing propagatory cell in each unchanged, flattened cell on periphery of the embryo (b) and (c) forming membrane.
- FIG. 31. Growing embryo of first generation redia (within sporocyst) showing propagatory cell with ectosomes, located at posterior end. This figure should be compared with figs. 32 and 33.
- FIG. 32. Growing embryo of second generation redia showing propagatory cell at posterior end.
- FIG. 33. Growing embryo of cercaria showing propagatory cell at posterior end.
- FIGS. 34-39 inclusive. Various stages of developing embryo of first generation redia showing propagatory cell in repeated unequal division. Note that the ectosomes in figs. 37, 38 and 39 do not pass into the smaller somatic cell.
- FIGS. 40 and 41. Embryos of cercaria and second generation redia respectively showing propagatory cell in unequal division.
- FIG. 42. Young miracidium inside the egg showing propagatory cell at posterior end of body. A portion of vitelline membrane (vm) is shown at one side of the body.



PLATE II

fate of this body is unknown; it may have broken down into fine granules dispersed in the cytoplasm.

Embryo: The two cells formed by the second cleavage now divide several times, while the propagatory cell remains unchanged. In the resultant cell mass (fig. 30a) four types of cells can be distinguished, viz., the single propagatory cell, cells with a large, ellipsoidal, lightly staining nucleus and a large nucleolus, cells with a small, lightly staining nucleus and small nucleolus, and cells of a size similar to the last mentioned, whose nuclei are filled with heavily staining, coarsely granular threads but no nucleolus. In the last three types of cells, the cytoplasm stains faintly, and the cell boundaries are rather indistinct.

With further divisions the cells of three types multiply; the propagatory cell, however, remains unchanged at one end of the cell mass opposite the group of large cells with ellipsoidal nuclei. Cells of the third type are scattered between those of the first two types and most of the cells of the fourth type are arranged at the periphery of the cell mass.

Now some of the small lightly stained third type cells separate from the main cell mass (fig. 30a), wander between the yolk cells (not shown in the figure), and arrange themselves next to the egg shell to form a vitelline membrane.

Later the propagatory cell divides. One of the daughter cells retains the characteristics of the parent cell, while the other becomes a somatic cell that gives rise to cells which differentiate later into muscles and glands. The cell mass at first spheroidal gradually becomes elongated and forms the young ellipsoidal miracidium (fig. 42).

Miracidium: At the periphery of the young miracidium there is a layer of cells with clear cytoplasm and well-stained, distinct nuclei (fig. 42). These cells later become flattened and their nuclei stain poorly and become less apparent. From these cells are later derived the ciliated epidermal plates. At the posterior end of the young miracidium the propagatory cell (fig. 42_p), though smaller after repeated divisions, still retains its original characteristics with large rounded nucleus, conspicuous nucleolus, fine chromatin granules and densely granular cytoplasm. This propagatory cell divides into germ cells which are destined to give rise to the next generation, i.e., rediae. In the newly hatched miracidium there are several germ cells (figs. 43, 44). The body cavity is formed but not clearly seen.

Discussion: Leuckart (1882) stated that germ cells are embryonic cells whose function is limited to reproduction. Schauinsland (1883) believed that by the epibolic process of development the cells of the embryo of the miracidium are separated into two kinds, ectoderm and endoderm. The former develops into the body covering and the ciliated membrane. Some of the endodermal cells are differentiated into the epithelium and the digestive tract, while some, as yet undifferentiated, remain as germ cells.

Goldschmidt (1905) in *Zoogonus mirus* found in the center of the embryo a large cell the "Entodermzelle," from which as a result of several divisions two groups of endodermal cells are formed. One group forms the middle gut; the other group develops into germ cells containing heavy plasma and large nuclei with fine chromatin granules.

Kathariner (1904) in *Gyrodactylus elegans* found that the first cleavage was equal, and designated the resulting cells "a and b." Cell "b" continues to divide equally while cell "a" divides unequally and its daughter cells divide unequally. When the miracidium is in the sixty-celled stage there persists one large cell which then gives rise by division to two cells resembling the "a and b" cells of the first cleavage. One of these divides equally and the resultant cells become somatic cells. The other gives rise by cleavage to several embryos, that is, it is a germ cell.

Ishii (1934) found that the first cleavage of *Fasciolopsis buski* was unequal. One cell with a darkly staining nucleus he called the propagatory cell; the other with a lightly staining nucleus but more abundant protoplasm he called the ectodermal cell. This propagatory cell does not divide until after three days of incubation when all other cells have divided several times. From the ectodermal cell originate the connective tissue cells, the vitelline membrane, the nervous system, and the excretory system. From the propagatory cell two cells are formed. One of these, the germ cell, multiplies by division while the other gives rise to cells which differentiate into the gut, muscle, and the cephalic gland.

These investigators are in agreement that the germ cell in the embryo can be traced back to the first cleavage stage. In this respect the history of the germ cell in the embryo of *Paragonimus* is the same.

Sporocyst: During penetration into the snail, the miracidium casts off the ciliated membrane and becomes an elongated sac or sporocyst which becomes filled with germ cells (fig. 45). In the young sporocyst these are not yet distinctly separated from the body cells, on account of the crowded condition of the cells and the indistinctness of the body cavity. The sporocyst grows more in length than in width. Elongation takes place on the sides of the body where the body wall gradually becomes distinct while the anterior and posterior ends are still left in their original thickened condition.

When the body wall shows clearly, the cavity becomes larger and shows distinctly between the germ cells and the body wall at the anterior end and the sides. But, posteriorly, a portion of the body wall is still in loose contact with the group of germ cells (fig. 50). In this group the newly differentiated cells keep on multiplying and may draw their nourishment from the body wall with which they are in contact.

The body wall at first is rather thick with clear spherical nuclei containing deeply staining nucleoli. But when the sporocyst matures and

elongates, its body wall becomes thinner and its cells have spindle-shaped nuclei. Later when the cavity of the sporocyst is filled with embryos and young rediae of the first generation, the body wall becomes a thin membrane with indistinct nuclei which may sometimes appear as small black spots.

In the body of the sporocyst are deeply staining stellate muscle cells whose branches extend toward the periphery of the body. Sometimes if there are several cells of the same kind close together, the branching processes anastomose. Another kind of cells somewhat resembles the muscle cells but they have only one slender cytoplasmic process directed toward the periphery of the sporocyst. These cells may be glandular cells as were described by Maclaren (1903). Haswell (1903) in a sporocyst of *Echinostomum* saw the same kind of cells secreting globules. Both muscle and glandular cells are easily mistaken for germ cells because of their appearance and location.

Germ cells: Germ cells, as already mentioned, were present in the body of the miracidium and these remain when the latter becomes a sporocyst. The number of germ cells is increased only by division of the original germ cells and not by division of cells in other parts of the body.

The group of germ cells shows a series graded in differentiation, multiplication, and growth from the posterior to anterior end where the formation of the germ balls takes place. The germ cells stain lightly and adhere loosely. Although sought diligently, no maturation processes have been observed in the sporocyst. By mitotic division the full grown germ cell

EXPLANATION OF PLATE III

- FIGS. 43 and 44. Miracidium, hatched, showing germ cells in body cavity.
- FIGS. 45, 46 and 47. Comparison of young sporocyst (fig. 45) in tissue of snail, young first generation redia (fig. 46), and young second generation redia (fig. 47) showing germ cells in body cavity of each.
- FIG. 48 First generation redia farther advanced in growth. Note group of germ cells in body cavity. Compare with figs. 49 and 50.
- FIG. 49. Posterior end of a fully grown second generation redia showing group of germ cells and germ balls (gb).
- FIG. 50. Posterior end of a fully grown sporocyst showing group of germ cells and germ balls.
- FIGS. 51, 52, and 53. Embryos respectively of second generation redia (fig. 51), of first generation redia (fig. 52) and of cercaria (fig. 53). All three embryos are in the differentiating stage. Note the germ cells in body of each.
- FIG. 54. Young cercaria showing early genital primordium, excretory bladder (bl), tail and pharynx (ph).
- FIG. 55. Growing cercaria showing further development of genital primordium, bladder, and tail.
- FIG. 56. Posterior end of a fully grown cercaria showing primordium of both male and female reproductive organs.
- FIG. 57. Spermatozoa not yet free from the central cytoplasmic mass.



PLATE III

divides rapidly to form a germ ball. The cells in the body wall gradually degenerate when the sporocyst becomes older; and the germ cells at the posterior end cease their activity apparently for lack of nourishment.

Discussion: Moulinié (1856), Leuckart (1882), Hofmann (1899), Haswell (1903) and Reuss (1903) thought that the sporocyst multiplies by binary division. Their arguments were carefully reviewed by Ssinitzin (1909) who summarized his conclusion thus: "Es gibt bei den digenetischen Trematoden keine ungeschlechtliche Fortpflanzung weder in Gestalt von Teilung noch als exo- oder endogene Knospung." In *Paragonimus* multiplication of sporocysts does not occur nor has it been observed by recent investigators in other species.

Regarding the origin of germ cells in the sporocyst, investigators have held various opinions. Leuckart (1879) found that at the posterior end of the sporocyst of *Fasciola hepatica* there was no clear contrast between the body cells and the germ cells because the former were not much differentiated and the latter retained their embryonic character. He meant that the germ cells were already there in the sporocyst. Looss (1892) thought that the sporocyst wall in *Amphistomum subclavatum* has the power of producing germ cells, but when the sporocyst becomes older, the producing area is localized in the posterior part. Thomas (1883) thought that the germ cells proliferate from the epithelial lining of the body cavity, while Biehinger (1884) stated that the budding of the germ cells in the old sporocyst takes place only at the posterior end.

Haswell (1903) considered that the embryos in *Echinostomum* developed from an "ovary" which projected into the cavity at the posterior end of the sporocyst. Reuss (1903) in *Distomum duplicatum* found germ cells appearing in all places in the wall from the cells of which the germ cells were thought to be derived. Tennent (1906) mentioned two methods of producing germ cells in the branching sporocyst of *Bucephalus*, by "scattered production" and by "localized production" from the blunt process at one end of the body.

Cary (1909) thought that in the young sporocyst of *Diplodiscus temperatus* the "egg cells" come from the body wall and in the older ones from the "localized ovary." He claimed to have found maturation division. Ssinitzin (1909) mentioned two kinds of ovary, the "diffused ovary" in the young sporocyst and the "circumscribed ovary" in the older sporocyst. The latter was again divided into the "stationary ovary" which was closely united with the body wall and the "erratic ovary" which projected into the cavity. Faust (1917) thought that he had found formation of polar bodies in the redia of a Holostome (*Cercaria flabelliformis*). Kathariner (1920) found that the germ cells are derived directly from the fertilized egg and remain independent in the body.

Brooks (1930) thought that the germ cells come from the primordial germ cell which is set aside at a certain time after the fertilized egg begins cleavage. Faust and Hoffman (1934) stated that the germ ball proliferates from the germinal epithelium lining the wall of the sporocyst.

In *Paragonimus*, the germ cells found in the sporocyst trace back to germ cells in the miracidium. There is no "scattered production" of germ cells, nor is there a "diffused ovary" as stated by Tennent and by Cary. When the sporocyst becomes older, the body cavity enlarges and there is no "stationary" or "erratic ovary" as Ssnitzin claimed to find. In place of these there is only a group of germ cells remaining at the posterior end of the body. No maturation stage has been observed.

THE REDIAL GENERATIONS

The first generation rediae develop in the body cavity of the sporocyst from the germ cells at the anterior end of the germ group, and they pass through successive stages from germ cells to germ balls, and finally to rediae. The more advanced rediae are found at the anterior end of the sporocyst. The development of a first generation redia was followed from a single germ cell through the germ-ball stage, to the formation of an adult redia. No maturation stages comparable to those found in bisexual development could be found.

The germ cell and its cleavage: A full grown germ cell in the sporocyst is spherical and has a deeply staining cytoplasm and a large clear nucleus containing a large, darkly staining nucleolus (fig. 26c). Two spherical darkly staining bodies are located in the cytoplasm near the nucleus at opposite sides. They may be the "ectosomes" that Nachtwey (1925) believed to be identical with the "Membrankörper," and which are also found in the propagatory cell in the first cleavage of the fertilized ovum of *Paragonimus* (fig. 27a). Extensive observations reveal that these "ectosomes" appear temporarily when the germ cell is full grown and that they disappear during the late telophase.

The early division of these germ cells (figs. 27b, 28b) are similar to the corresponding stages (figs. 27a, 28a) found in the embryo of the miracidium. These divisions result in the formation of four cells (fig. 29b), each differing in appearance from the others. These are a propagatory cell, a large lightly staining cell, a small lightly staining cell, and a heavily granular cell.

Germ ball: Succeeding divisions affect the last three types of cells; the propagatory cell alone remains without change (fig. 30c). When the germ ball consists of about ten cells, one of the large lightly staining cells shifts to the periphery of the mass and there becomes flattened. This cell makes its first appearance in the germ ball at the future anterior end of the em-

bryo, opposite the propagatory cell. The flattened peripheral cell later divides several times to form a membrane with several nuclei in a continuous cytoplasm.

Embryo: All the cells in the germ balls, except the propagatory cell, continue to divide and the germ ball grows (fig. 31), becomes ovoid, and its ends are differentiated into anterior and posterior. This now can be called the embryo of the first generation redia. The propagatory cell at the posterior end divides unequally, the larger cell (fig. 31_p) retaining the characteristics of the original germ cell; the smaller cell (fig. 31) with little cytoplasm, divides into cells of equal size, and these give rise to those somatic cells which are later differentiated into the muscles and glands.

The propagatory cell continues dividing unequally during the growth of the embryo (figs. 31, 34, 35, 38, 39, 37, 36), the smaller cell always contributing to the mass of somatic cells and the relatively large propagatory cell (figs. 31_p, 34_p, 35_p, 38_p, 39_p, 37_p, 36_p) retaining the characteristics of the early germ cell and remaining near the posterior end of the embryo. This propagatory cell gradually becomes smaller as a result of repeated division until embryonic differentiation occurs.

The "ectosomes" in the early germ cell appear again in the propagatory cell (figs. 38_p, 39_p, 37_p) but disperse as fine granules during the late telophase when the cell divides unequally. During cell division the "ectosomes" remain in the larger cell, which thus maintains its original ectosomal content regardless of the number of divisions.

When in embryonic development the period of differentiation begins the propagatory cell divides equally to form germ cells (fig. 52g). These newly formed and least differentiated germ cells are small, rather darkly staining and scarcely distinguishable from the surrounding somatic cells. Soon, however, some of the germ cells begin to grow and are then easily differentiated from the body cells by their large clear nuclei and heavily granular cytoplasm. A large number of the germ cells grow so that when the gut is formed and the body cavity appears, the group of germ cells is clearly seen in the body cavity.

Young first generation redia: As the first generation redia grows these germ cells are left in a group at the posterior end (figs. 46, 48) more or less in contact with the body wall. They show gradation in differentiation, multiplication and growth, from the posterior to the anterior end of the germ group where the germ balls are formed.

Second generation rediae: The second generation rediae develop from a single germ cell within the body cavity of the first generation rediae in a manner strictly comparable with the development of the first generation rediae as related above. Chromosomes were counted during the division of the germ cell but no reduction divisions were seen nor were polar bodies formed. The fully developed germ cell (fig. 26b) including ectosomes is

similar cytologically to the germ cell found in the sporocyst. In the mitotic division of the germ cell two unequal cells are formed. As before the smaller of these is the propagatory cell and the larger is the ectodermal cell.

The early embryonic stage and its development (figs. 27c, 30b, 32) are similar to those giving rise to the first generation redia (figs. 27b, 30c, 31). So also the differentiating stages (figs. 41, 51) of the second generation redia are similar to that of the first generation redia (figs. 38, 52).

As the second generation redia grows (figs. 47, 49) the body wall becomes more definite and the musculature better developed, while the body cavity provides more space for the embryos of the cercariae.

Discussion: Thomas (1883) believed that the germinal cell which gives rise to the redia is already present in the embryo but at the same time he believed that additional germ cells come from the body wall. The first part of this statement, but not the second, is true for *Paragonimus*.

Reuss (1903) in *Distomum duplicatum* found in the germ balls of the three-, five-, seven- and twenty-five-celled stages a large cell possessing the characteristics of the original germ cell, but he could not follow it further because of the increasing numbers of other kinds of cells.

In *Paragonimus* the propagatory cell that gives rise to the germ cell is present in the embryo. It must be followed closely in order to find it among the other cells from which it is distinguished chiefly by its staining qualities.

The earliest germ cells found by Brooks (1930) were the "antecedent" germ cells that were already in the body cavity of both the sporocyst and the redia. They correspond to the fully grown germ cells in *Paragonimus*. The earliest germ cells found by Cable (1934) were the least differentiated germ cells in the body of both the redia and cercaria. Neither Brooks nor Cable traced the germ cells back as far as the propagatory cell. Cable found it difficult to follow the germ cell in the germ-ball stage. In *Paragonimus* the germ balls were studied from serial sections in which the differentiated germ cells could be observed.

In the formation of the germ ball, the two cells resulting from the first cleavage were called "macromere" and "micromere" respectively by Brooks (1930) and Cable (1934). In *Paragonimus* these terms are unsuitable because the size of cells is neither constant nor reliable. Such terms do not indicate the characteristics and the subsequent history of the cells.

Brooks (1930) believed that the multiplication of rediae and cercariae is accomplished by dissociation of germ masses which develop from "antecedent" germ cells. Cable (1934) found no such dissociation of germ masses in *Cryptocotyle lingua*, nor did it occur in *Paragonimus*. In the latter the germ cell develops directly into an embryo instead of into a germ mass.

In the germ ball and the embryo of *Paragonimus* there are no degenerated nuclei as Cable found. When the cells in the embryo are active in division, the telophase stage and the newly formed small cells always appear

as intensely staining small masses of chromatin material which may be easily mistaken for degenerated cells or yolk materials.

Hegner (1914) who investigated the "Keimbahn determinants" in insects and reviewed the literature relating to these structures in other groups of animals found them appearing at a certain time and place. They later disintegrate and disappear so that they could not be seen throughout the entire germ cell cycle, although the cytoplasm which contained them was continuous. After successive division the germ plasm was entirely separated from the somatic plasma. In *Paragonimus* the ectosomes in the germ cells and in the propagatory cells are similar in their behavior to the "Keimbahn determinants" of Hegner and their presence indicates that the germ plasm is continuous throughout the development of the sporocyst, redia, cercaria, and the adult.

CERCARIA

Cercariae develop in the body cavity of the second generation redia from fully grown germ cells at the anterior end of the germ cell group. These germ cells divide to form germ balls which develop into cercariae. The development of a cercaria was followed from a single germ cell through the early cleavage stages and germ-ball formation to the adult cercaria. Cell division was carefully studied and the number of chromosomes were counted without finding any stages of maturation or the formation of a polar body. The development of a germ cell and its cleavage to form a cercarial germ ball is clearly similar to the development of the germ cells and the germ balls which give rise to the two generations of redia.

As in the previous generations, the propagatory cell in the developing cercaria is present in the germ ball and embryo (figs. 33, 40) and divides equally when the embryo reaches the stage of differentiation, so as to form germ cells (fig. 53) which are small and relatively darkly stained. From this time the further development of these germ cells within the cercariae differs from those in the previous generations. These germ cells do not advance in differentiation and growth, but multiply by division to form a mass of numerous small darkly staining cells, the genital primordium (figs. 54, 55).

During this time the tail and excretory bladder begin to form at the posterior end of the body. The first indication of the bladder is a group of cells with clear nuclei arranged in a cord along the mid-line and extending anteriorly a short distance from the posterior end of the body. Later a lumen is formed inside the cord of cells. As the excretory bladder develops, its anterior end grows nearer to the genital primordium from which some cells shift to the two sides of the bladder to form the testes (fig. 56), while those remaining at the anterior end of the bladder form the primordium of the female reproductive organs (fig. 56). The further formation and

differentiation of the reproductive organs takes place after the cercaria passes into the metacercaria stage.

Cable (1934) identified a number of small opaque nuclei anterior to the excretory bladder as the germ cells but was not able to trace them back to the germ-ball stage.

DISCUSSION

From the above observations in *Paragonimus*, germinal lineage is shown without interruption. This agrees with the view expressed by Dollfus (1919) that two elements, somatic and germinative, come from a segmented egg, and the germinative element multiplies in the sporocyst and redia, and then gives rise to somatic and germinal elements of the next generation. Therefore the theory of germinal lineage with polyembryony applies to this species of trematode.

Brooks (1930) and Cable (1934) found that the theory of germinal lineage with polyembryony was applicable to the trematodes that they had studied. However, the results of their observations and mine on *Paragonimus* are not in entire agreement. According to Brooks, germ masses were found in the sporocyst and redia. This process of embryonic development resembles that occurring in the parasitic Hymenoptera described by Marchal (1904). Brooks's explanation for the formation of the germ mass is that the germ cells in the sporocyst and redia undergo precocious cleavage while the somatic development does not stop until the germ cells attain a sufficient physiological age and morphological differentiation.

Cable (1934) was unable to find formation of germ masses in *Cryptocotyle lingua*. Nor have I found in *Paragonimus* formation and dissociation of the germ masses. From Brooks's (1930) description of the sporocysts and rediae, which are the only stages that he studied, it seems that most of his specimens were too old for the study of the early history of the germ cells. The sporocysts and rediae that he described were mostly well formed, and perhaps corresponded to the stage in *Paragonimus* in which the body wall was well formed and most of the germ cells already developed into young embryos.

Although Cable (1934) made no direct observations on miracidium and sporocyst, he believed that germinal lineage occurs in them. From his observations on the redia, Cable thought that in them, as in the sporocyst, the somatic cells do not contribute to the reproductive cells. He concluded that "germinal lineage occurs with certainty in the redia and probably also in the sporocyst since it is still nearer the fertilized egg than is the redial generation." His view of germinal lineage in the miracidium, sporocyst and the redia is supported by my observations in *Paragonimus*.

As Cable (1934) failed to find the germ cell in the germ ball and in the early stages of the young cercaria, he thought that germinal lineage is

interrupted there. He tried to give some explanation on the basis of Brooks's hypothesis (1930), and thought that the interruption of the germinal lineage is due to the decreasing intensity of the germinal precocity in the germ ball. Some of the cells in the germ ball were thought to be equipotent or totipotent to both the somatic and germinal line and later give rise to elements of both. After the genital anlage appears the germinal lineage becomes re-established. It is my belief that if Cable (1934) had had more material for his study, and had followed further the development of the embryo, he would not have needed the explanation used by him for the stages which he was unable to observe.

As already mentioned in the early part of this paper, in the study of the germ-cell cycle of trematodes, the method and material are very important. Brooks (1930) used the method of comparative study involving the sporocysts or rediae of twenty species of trematodes. Since the sporocyst or redia is only a generation in the life cycle of the trematodes, the complete germinal cycle cannot be definitely determined from these observations. For this reason Woodhead (1931) and Cable (1934) preferred to study intensively the germinal cycle in the complete life history of a single species. However, they were unable to observe some generations or certain stages for lack of material. It is recalled that Cable (1934) stated, "The succeeding divisions of cells of the germ ball are exceedingly difficult to follow . . ."

In *Paragonimus* the germ cell cycle was followed from the adult to cercaria (the larva of the adult). In the rich material studied, no interruption of germinal continuity was observed. The development of the germ cell in *Paragonimus* was traced from stages much earlier than those in which Brooks (1930) found the antecedent germ cells, and far later than those in which he found the formation of the embryos. I was unable to find the formation and dissociation of germ masses as described by Brooks (1930). In my method of study in which a large amount of material was used it is not likely that any significant developmental stages were missed.

The diversity of observations made by Brooks (1930), Woodhead (1931), and himself caused Cable (1934) to state that the germinal cycle of trematodes can only be explained by a hypothesis based on the phylogeny of the Digenea. He suggested that the ancestors of this group were sexually mature in the mollusc and completed the cycle in that host; and that with the evolution of trematodes, sexual phenomena were gradually lost, and were replaced by polyembryony with or without formation of germ masses in larval multiplication. He assumed that *Cryptocotyle lingua* had been modified to an intermediate extent. This hypothesis appears sound in explaining the different observations of Brooks (1930) and Woodhead (1931), but it is not likely to explain the difference between the observations of Brooks and Cable (1934).

However I am in accord with Cable (1934) who states that conclusions concerning reproduction in the entire group of trematodes must not be drawn until more data can be obtained from a large number of complete life histories in which the germ cell cycle has been worked out from adult to adult.

SUMMARY

The germ cell cycle in *Paragonimus* has been followed in all developmental stages from adult to cercaria (larva of adult). Oogenesis, spermatogenesis, maturation and fertilization were studied in the adult as a basis for a study of the germ cells of the other generations. The chromosome number in *Paragonimus* was found to be sixteen.

1. Cleavage of a fertilized ovum gives rise to cells of two kinds, somatic and propagatory (stem). Somatic cells form the body of the miracidium. The single propagatory cell is retained within the body of the embryo and undergoes several unequal divisions, the smaller of the resulting cells contributing to the somatic cells of the miracidium, the larger ones dividing to form the germ cells during the period of differentiation of the embryo.

2. When the miracidium becomes a sporocyst after penetrating into the snail host the germ cells are carried within the body cavity where they develop into germ balls which become the first generation of redia.

3. The development of the first and second generation rediae which was followed step by step from a single germ cell to the fully developed redia is similar to the development of the miracidium.

4. Nothing has been found to indicate that germ cells are derived from the cells of the body wall of the sporocyst and redial generations.

5. An ovary was not observed in either sporocyst or redial generations nor was there any evidence of parthenogenesis or of fertilization in these generations.

6. Germ cells in the sporocyst and redial generations develop directly into embryos and these into adults without the formation of germ masses and their subsequent dissociation.

7. Ectosomes are visible in both the early germ cell and the propagatory cell during certain periods.

8. The germ cells in the body cavity of daughter rediae develop into cercariae within which the process of embryonic development and formation of germ cells is similar to that in the preceding generations. But in their later development the germ cells in the cercariae multiply to form genital primordia instead of forming embryos as before.

9. The germ cells in the genital primordium of the cercaria differentiate into male and female germ cells during the metacercaria stage.

10. Germ cells which differentiated in the metacercaria undergo maturation in the hermaphroditic adult.

CONCLUSION

The theory of germinal lineage with polyembryony can be applied to *Paragonimus*, in which the germ cells in the adult are differentiated into oogonia and spermatogonia. After maturation and fertilization, the ovum in the course of cleavage and the formation of an embryo delivers the germ plasm to one of the daughter cells, the propagatory cell. Segregation of germ plasm continues until the beginning of the differentiation of the embryo, when the propagatory cell divides into germ cells with equally distributed germ plasm.

These germ cells multiply and grow, and each of the descendant cells develops without formation of germ masses or their later dissociation or any maturation process into an embryo in which the germ plasm is segregated as in the preceding generation. This process occurs in the sporocyst and both redia generations but stops with the formation of the cercaria.

In the cercariae which are the larvae of the sexual generation, the germ cells multiply to form the genital primordium in which the cells differentiate into male and female germ cells whose descendants finally undergo maturation in the adult.

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